Genetics of alcohol use and liver enzymes

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CHAPTER 1

GENERAL INTRODUCTION

Alcohol use is a serious risk factor for disease. Although beneficial effects of alcohol use have been reported, the total net effect on health is disadvantageous. Light to moderate levels of alcohol use have been related to lower risks for coronary heart disease (CHD) and type 2 diabetes, but for individuals who drink heavily (even occasionally) relative risks are higher. In Western countries, 8-12% of (in)direct aversive health effects are due to alcohol use (calculated in DALYs, disability-adjusted life years) in men, while this is 0.5-3% for women (Rehm et al. 2003). Most alcohol-attributable deaths result from cancer, cardiovascular disease, liver cirrhosis, diabetes and injury (Rehm et al. 2003). The average daily volume of alcohol intake (ADV) associated with increased mortality risk lies around 35-45 grams (~3 glasses). Risk of disease already starts to increase at an ADV of 25 grams (for hypertension, liver cirrhosis, chronic pancreatitis, various cancers), although for some diseases the ADV needed is much higher (>60 grams for type 2 diabetes, stroke; >89 grams for CHD, i.e. >4 and >6 glasses) (Dawson 2011). Other possible negative effects of alcohol use encompass alcohol use disorders (AUD; e.g. addiction), mental health problems, disruption of social relationships, loss of work productivity, as well as aggression, violence, and legal problems (NIAAA 2000).

The vast majority of people consumes alcohol, at least occasionally, which is likely to be explained by the positive effects of alcohol use on stress reduction, mood elevation, relaxation, and increased sociability (NIAAA 2000). Based on data from the World Health Organization, in the Netherlands, 82% of men and 64% of women aged 15 or older consumed alcohol in the past year. The prevalence of current drinking is even higher in the UK (90% and 82%), Germany (96% and 95%), Canada (81% and 74%) and Australia (88% and 80%), but lower in the USA (72% and 59%). The prevalence of heavy drinking however is highest among the Dutch. Twenty percent of Dutch men report to drink heavily (>60 grams of alcohol weekly at least once a week, i.e. >4 glasses) and five percent of the women. Estimates for the other countries are: 10% and 3% (Australia), 13% and 3% (USA), 14% and 2% (Germany) and 16% and 4% (Canada) (no estimates are reported for the UK) (World Health Organization 2011). Data from the Netherlands Twin Register (NTR) indicate that 9-17% of men and 3-11% of women drink heavily (>21 and >14 glasses per week respectively for individuals aged ≥18) (Geels et al. 2013). Given the high prevalence of alcohol use and heavy drinking in combination with the multitude of disease associations, understanding the causes of individual differences in (problematic) alcohol use is important.

1. Drinking guidelines and definitions of (problematic) alcohol use

Drinking guidelines on safe alcohol use are based on its harmful consequences for health and vary across countries (Health Counsel of the Netherlands, 2006; Dawson et al., 2011) as well as within countries across advisory organizations (Health Counsel of the Netherlands, 2006). Differences can partly, but not entirely, be explained by variation in the standard drink size between countries (Dawson et al., 2011) that ranges from 8 grams (UK) to about 14 grams (USA) (Dawson 2011; Health Counsel of the Netherlands 2012). Defining a standard drink size as containing 10 grams of alcohol, the Health Counsel of the Netherlands recommends daily alcohol use not to exceed two glasses of alcohol for men (≤20 grams) and one glass of alcohol for women (≤10 grams). These daily limits do not refer to an *average* daily volume of alcohol intake (e.g. heavy weekend drinking with five days of non-drinking), but emphasize that alcohol use should be kept within daily limits, each day. Countries generally recommend complete abstinence for youth and women that are (trying to get) pregnant, or breastfeeding (Health Counsel of the Netherlands 2012).

Definitions of problematic alcohol use can be separated into those that focus on the amount of alcohol consumption (e.g. excessive drinking) and those that refer to the negative physical and social effects of alcohol use (AUDs) (Fiellin et al. 2000). Excessive alcohol use is defined as drinking more than three alcoholic beverages a day *on average* for men (ADV >30 grams) and more than two alcoholic drinks *on average* for women (ADV >20 grams) (Health Counsel of the Netherlands 2012). Excessive drinking is known to put individuals at risk for alcohol-induced health-related consequences (therefore also known as 'hazardous drinking') (Fiellin et al. 2000). AUD diagnoses are based on manuals as the Diagnostic and Statistical Manual of Mental Disorders (4th edition; DSM-IV) (American Psychiatric Association 2000) and the International Classification of Mental and Behavioral Disorders (10th edition; ICD-10) (World Health Organization 1992). The DSM-IV (American Psychiatric Association 2000) distinguishes two types of AUDs: alcohol dependence and alcohol abuse. Alcohol dependence is defined as a maladaptive pattern of alcohol use, leading to clinically significant impairment or distress, broadly characterized by physical dependence (tolerance, withdrawal symptoms), psychological dependence (craving), loss of control (exceeding intended amount; unable to cut-down use), and physical and social consequences of maladaptive alcohol use. The diagnosis of alcohol abuse can be seen as a lighter version or precursor of alcohol dependence. It can be defined as a maladaptive pattern of alcohol use, leading to clinically significant impairment or distress, that is manifested by interpersonal and social problems due to alcohol use, without having symptoms of dependence or loss of control (Van den Brink 2010).

Although the diagnosis of AUD does not require a specific level of alcohol consumption, excessive alcohol use is a predictor for developing AUD. Both the average volume of consumption and the frequency of heavy drinking are independently associated with the risk of AUD. Drinking five or more drinks at a day for men (≥70 grams) or four or more for women (≥56 grams), was associated with a higher incidence of AUD, and the frequency of drinking these amounts further increased this risk (Dawson 2011). To avoid the development of AUDs, some organizations recommend to avoid daily drinking (Health Counsel of the Netherlands 2012).

Several brief screening instruments have been developed to detect excessive alcohol use and alcohol use disorders, including the CAGE questionnaire (Ewing 1984), and the Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al. 1993). The AUDIT is developed to screen for excessive alcohol use that puts the health of individuals at risk. In addition to screening for excessive alcohol use it performs well in detecting alcohol dependence. Several shortened versions exist that show comparable screening properties (Reinert and Allen 2007). The CAGE, designed to detect alcohol dependence, is also valuable in detecting alcohol abuse, but less so in screening for excessive alcohol use. When combining the CAGE with alcohol consumption items on quantity and frequency, the performance in screening for excessive drinking is much increased (Dhalla and Kopec 2007; Berks and McCormick 2008). An overview of the measures for (problematic) alcohol use and other phenotypes that are used in this thesis is given in Chapter 2.

2. Alcohol metabolism and resulting tissue damage

Health-related consequences of alcohol use may, at least partly, be ascribed to effects of alcohol metabolism that primarily takes place in the liver. In humans, there are three pathways of alcohol oxidation. The bulk of ingested ethanol is oxidized by the alcohol dehydrogenase enzymes (ADH) which are present in the cytosol. At high alcohol concentrations and during chronic alcohol abuse, the cytochrome P450 isozymes, including CYP2E1, that are present in the endoplasmic reticulum, contribute to alcohol metabolism. This system is also called the microsomal ethanol oxidizing system (MEOS). A third, but minor, oxidative pathway is formed by the enzyme catalase that is located in the peroxisomes. Alcohol that is oxidized through these pathways results in the formation of acetaldehyde, a highly reactive and toxic byproduct that, with the enzyme aldehyde dehydrogenase (ALDH), is oxidized to acetate in the mitochondria (Zakhari 2006). The toxicity of acetaldehyde diminishes the capacity of the mitochondria to metabolize acetaldehyde, leading to higher levels of acetaldehyde in the mitochondria which contributes to alcohol-related tissue damage (Caballeria 2003). Other conditions that can result from alcohol oxidation and that can

induce tissue damage are fatty liver, oxidative stress, hypoxia (through increased oxygen consumption) and inflammation. See Figure 1 for an overview of the metabolic changes in the liver associated with alcohol metabolism (taken over from Callallería (2003)).

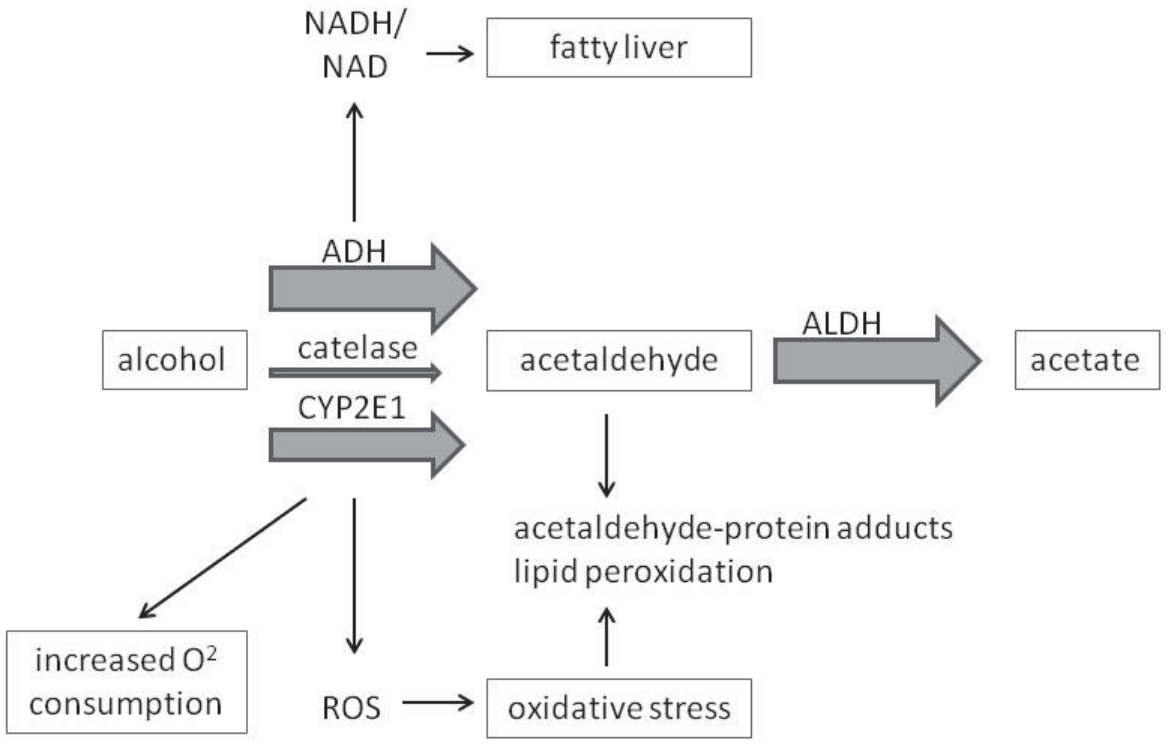

Figure 1 Metabolic changes in the liver associated with alcohol metabolism (taken over from Caballería, 2003)

The development of fatty liver, an excess of triglycerides in the liver, results from a change in the redox (*red*uction-*ox*idation) state in the cytosol and mitochondria. The conversion of ethanol to acetaldehyde by ADH and from acetaldehyde to acetate by ALDH, involves an intermediate carrier of electrons, nicotinamide adenine dinucleotide (NAD+), which is reduced to NADH. This results in an increased NADH/NAD+ ratio in the cytosol and mitochondria (Zakhari 2006). This redox imbalance leads to an excess of triglycerides in the liver by complex mechanisms that increase fatty acid synthesis, decrease hepatic lipoprotein secretion, increase mobilization of fatty acids from adipose tissue favoring their hepatic reuptake and decrease fatty acid oxidation (Caballeria 2003).

The oxidation of ethanol and acetaldehyde results in the generation of oxygen-containing free radicals, called reactive oxygen species (ROS). ROS are toxic because they react with

macromolecules including proteins, lipids and DNA, what can cause their degradation. A variety of enzymes as well as (non-enzymatic) anti-oxidants protect cells from ROS. A disturbance in the balance between ROS generating and protecting factors, resulting in an excess of ROS is called oxidative stress. The presence of certain metals in the cell, especially iron, promotes the interaction between some free radicals and thus catalyzes ROS. Chronic alcohol consumption increases iron levels in the body through increased iron absorption from food (a characteristic not limited to iron-rich red wine) and therefore ameliorates the toxic effects of alcohol on the body (Wu and Cederbaum 2003).

Ethanol tends to increase the oxygen intake by hepatocytes which can induce other liver cells in a state called hypoxia. Oxygen is needed for NADH (the reduced form of NAD+ that results from ethanol oxidation) to pass through the mitochondrial electron transport system in the mitochondria. During this process, H+ is removed from NADH, to obtain NAD+ that can be used again for ethanol oxidation. Oxygen is used to bind to the H+ molecules to form H_2O (Zakhari 2006). If liver cells that are close to the fresh blood supply (near the hepatic artery) take up increased amounts of oxygen, not enough oxygen may be left for liver cells that take up their oxygen supply later in the blood track (near the hepatic venule), leaving those cells in a state called hypoxia (Caballeria 2003). After chronic alcohol consumption, the liver cells near the hepatic venule are the first to show cell death (Cunningham and Van Horn 2003).

The immune system can be modulated by the effects of alcohol in several ways. One is the activation of the body's inflammatory response to the various biochemical metabolic effects that result from alcohol metabolism. Fatty acid accumulation in the liver induces synthesis of pro-inflammatory cytokines. Acetaldehyde can bind to proteins of various cellular membranes forming acetaldehyde-protein complexes that induce antibodies to bind to these complexes and destroy the hepatocytes that contain them. Death cells, for instance those that result from hypoxia or mitochondrial damage, also activate the inflammatory response (Wang et al. 2010). Second, long term alcohol exposure causes a change in the cytokine milieu resulting in a systematic inflammatory condition that can damage various tissues (Wang et al. 2010). Chronic alcohol consumption can cause leakiness of the digestive tract, which can result in the release of endotoxins in the blood that activate Kupffer cells that reside in the liver (Kovacs and Messingham 2002). These Kupffer cells stimulate the release of several inflammation-promoting cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-8. Kuppfer cells are a major source of ROS, further increasing possible tissue damage (Vidali et al. 2008). In addition, alcohol use is known to have immunosuppressive effects on both the innate and the adaptive immune system,

increasing the risk of infections (Molina et al. 2010; Kovacs and Messingham 2002). Finally, liver injury that results from inflammation can diminish the liver's ability to regulate inflammatory responses, which further ameliorates injury through the inflammatory response (Wang et al. 2010).

It should be noted that ethanol is also metabolized through non-oxidative pathways and can take place outside the liver as well. Non-oxidative pathways of ethanol metabolism include formation of molecules called fatty acid ethyl esters (from the reaction of alcohol with fatty acids) and formation of phospholipids that are known as phosphatidyl ethanol (Zakhari 2006). ADH activity is present in the stomach and colon mucosa. Marked gender differences have been observed for ethanol metabolism in the stomach. Gastric ADH activity is lower in women, which may be one reason why women are more susceptible to the toxic effects of alcohol. It is suggested that bacteria, that make up most of the flora in the human colon, can oxidize ethanol to acetaldehyde by bacterial ADH and subsequently to acetate by bacterial ALDH (Caballeria 2003).

3. Epidemiological research on the effect of alcohol use on fatty liver and oxidative stress

Epidemiological research on alcohol use supports the effects on liver injury described above, at least for heavy drinking. A longitudinal study showed that an increase in the quantity of alcohol consumed was associated with a higher risk of developing a fatty liver and a decrease in alcohol consumption with reduction of fat tissue within the liver (Bedogni et al. 2007). In most studies, a higher prevalence of fatty liver was observed among heavy drinkers (>40 grams ADV or alcohol abuse diagnosis) compared to individuals who drink less than 40 grams alcohol per day or abstainers (Hiramine et al. 2011; Bellantani et al. 1997; Pares et al. 2000; Chen et al. 2004; Fan et al. 2005). Studies among Japanese individuals consistently show that moderate drinking is associated with a lower prevalence of fatty liver when compared to abstaining (Yamada et al. 2010; Hiramine et al. 2011; Omagari et al. 2009; Gunji et al. 2009). This higher prevalence of fatty liver among abstainers could not be attributed to individuals who had stopped drinking because they received a diagnosis of fatty liver. Participants who had received the diagnosis of fatty liver during a previous examination were comparable in their level of alcohol use to participants who received the diagnosis of fatty liver for the first time (Hamaguchi et al. 2012). Furthermore, whereas for Japanese women, the prevalence of fatty liver among heavy drinkers was comparable to that among abstainers (Omagari et al. 2009; Hamaguchi et al. 2012; Yamada et al. 2010), for Japanese men, in some studies the prevalence of fatty liver among heavy drinkers was lower than that of abstainers (Hamaguchi et al. 2012; Yamada et al. 2010), although not in all (Omagari et al. 2009).

Effects of oxidative stress are detectable at both moderate and heavy drinking levels. Although the detection of alcohol-induced ROS generation in vivo is difficult (Wu et al. 2006), alcohol exposure has an effect on anti-oxidant levels and on surrogate markers of oxidative stress. Anti-oxidant carotenoid concentrations were lower in excessive drinkers (>25 grams ADV) than in abstainers (Sugiura et al. 2005). After drinking either beer, wine or spirits for 30 days (40 grams alcohol/day), levels of lipid peroxidation were increased and anti-oxidants levels decreased (Addolorato et al. 2008). Increased levels of oxidative stress, as measured by urid acid concentrations, were detected among normal weight excessive drinkers (>30 grams alcohol/day (Oliveira et al. 2010) as well as moderate drinkers (1-21 glasses alcohol/week) (Alatalo et al. 2009a) relative to abstainers (BMI <25). Note however, that in the study of Alatalo et al. (2009a), urid acid levels for women were comparable to those for abstainers (taking effects of BMI into account). Thus, the effect of oxidative stress by alcohol may be dependent on sex.

4. Liver enzyme levels and its relation to alcohol use

This thesis focuses on alcohol use and blood levels of three enzymes that give an indication of injury to the liver: gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Blood levels of these liver enzymes were originally proposed as biomarkers for detection of heavy drinking (Whitfield 2001), but, overall, their clinical value has proven to be limited.

GGT is a glycoprotein enzyme (a large molecule made up of both proteins and carbohydrates) that is situated on the membrane of cells in several tissues, including cells that are involved in bile production (Peterson 2004). It is involved in maintaining adequate levels of intracellular glutathione, the most abundant intracellular antioxidant, that are needed to protect the cell from oxidants that result from the metabolism of ethanol and other products (Whitfield 2001). Increases in GGT activity are thus expected during ethanol metabolism, to protect the cell from oxidative stress. Studies consistently show that GGT levels and the incidence of elevated levels is increased for heavy drinkers (>40 grams ADV) relative to abstainers as well as to moderate drinkers (Alatalo et al. 2009b; Chen et al. 2003; Ioannou et al. 2006; Nakanishi et al. 2000; Conigrave et al. 2002). The odds ratios associated with elevated GGT levels due to heavy alcohol use range from 3.85 to 18.4 (Scouller et al. 2000; Lee et al. 2001; Nakanishi et al. 2000; Higashikawa et al. 2005; Sillanaukee et al. 2000b; Steffensen et al. 1997; Arndt et al. 1998). Most studies report increased GGT levels for moderate drinkers in comparison to those for abstainers (Lee et al. 2001; Nakanishi et al. 2000; Higashikawa et al. 2005; Arndt et al. 1998; Liangpunsakul et al. 2010),

although in some studies GGT levels are similar to that of abstainers (Sillanaukee et al. 2000b; Steffensen et al. 1997). Whereas increasing levels of alcohol consumption are associated with increasing levels of GGT, its value as a marker of chronic alcohol use/abuse is limited. This is because an increase in GGT levels is not specific to heavy drinking (and conversely, not every heavy drinker has elevated GGT levels). Sensitivity estimates for GGT in detecting heavy drinking (>40 grams ADV) or a diagnosis of alcohol abuse or dependence, ranged from 7% to 65% for males; and from 7% to 61% for females, at a specificity of 90-95%. For studies that estimated the specificity to be 80-85%, the sensitivity was estimated to be around 39%-71% (Salaspuro 1999; Allen et al. 2000; Sillanaukee and Olsson 2001; Alte et al. 2004; Aertgeerts et al. 2001; Conigrave et al. 2002). The large differences in the sensitivity/specificity estimates can be explained by differences in the definition of problematic alcohol use that were used (>40/ >60/ >80 grams ADV/current/lifetime disorder of alcohol abuse/dependence), as well as by differences between studies in the prevalence of heavy drinking. Lower sensitivities are expected, and were detected, among the general population, in which the prevalence of heavy drinking is lower, than in medical wards and primary health care settings (Salaspuro 1999).

The association of alcohol use with aminotransferase levels (AST and ALT) is weaker than that with GGT. Blood levels of AST and ALT are markers of hepatocellular injury, that increase when damage to the liver cell membrane leads to increased leakage of AST and ALT into the circulation (Pratt and Kaplan 2000). ALT is present in several organs and muscle, but mostly in the liver and is therefore more specific to liver injury than AST which is found in the liver and skeletal muscle (Hannuksela et al. 2007), but also in the heart, and kidneys (Pratt and Kaplan 2000). An AST/ALT ratio of ≥2:1 is reported to be suggestive of alcoholic liver disease (Hannuksela et al. 2007; Sorbi et al. 1999; Nyblom et al. 2004). Although there is evidence that AST levels are increased by heavy drinking, they are not or only to a small degree increased by moderate levels of alcohol use. One study detected higher AST levels among moderate drinkers than among abstainers (Liangpunsakul et al. 2010), but another did not (Alatalo et al. 2009b). In three other studies the incidence of elevated AST levels among moderate drinkers was comparable to that of abstainers (Lee et al. 2001; Steffensen et al. 1997; Arndt et al. 1998). Heavy drinking was associated with increased (incidence of elevated) AST levels, compared to abstainers, in most studies (Arndt et al. 1998; Alatalo et al. 2009b; Chen et al. 2003; Conigrave et al. 2002), but not in all (Lee et al. 2001) and in one study AST levels were elevated only among women (Steffensen et al. 1997). The sensitivity of AST to detect problematic alcohol use has been estimated around 11-45% at a specificity of 90-95% and around 50% at a specificity of 80% (Salaspuro 1999; Conigrave et al. 2002).

There is some suggestion that ALT levels are increased by heavy alcohol use, but clear evidence is absent. Moderate drinking was associated with elevated ALT levels in two studies (Alatalo et al. 2009b; Ioannou et al. 2006) (in Alatalo et al. (2009b) for men only), but not in three others (Lee et al. 2001; Steffensen et al. 1997; Arndt et al. 1998). Of four studies that looked at the effect of heavy drinking (Lee et al. 2001; Steffensen et al. 1997; Alatalo et al. 2009b; Arndt et al. 1998), two studies detected higher ALT levels or an increase in elevated levels among heavy drinkers compared to abstainers (Arndt et al. 1998; Alatalo et al. 2009b). One study did not detect such an effect (Lee et al. 2001), and another only among women (Steffensen et al. 1997). The sensitivity of ALT to detect problematic alcohol use is estimated between 35% and 47% at a specificity of 85% (Salaspuro 1999).

Liver enzyme levels are associated with disease and mortality. GGT, ALT and AST all strongly predict liver disease and liver-related mortality (Ruhl and Everhart 2009; Kazemi-Shirazi et al. 2007; Hyeon et al. 2004; Lee et al. 2008), with GGT and ALT showing relations with other diseases also. Strong associations have been found for GGT and ALT with type 2 diabetes (Fraser et al. 2009), and for GGT with cardiovascular disease (Targher 2009; Fraser et al. 2007) as well as cancer and chronic kidney disease (Targher 2009). These disease associations cannot be solely explained by the negative effects of alcohol use alone, since most disease associations are still present when taking alcohol use into account (Targher 2009; Ruhl and Everhart 2009; Fraser et al. 2009).

In clinical practice, GGT, ALT and AST are used as markers of liver injury. To explain their role in other disease, GGT and ALT have been proposed as surrogate markers of fatty liver (Targher 2009; Schindhelm et al. 2006) and GGT as marker of oxidative stress as well (Lee et al. 2004). The role of GGT and ALT as marker of fatty liver comes from the positive correlations of GGT and ALT with liver fat content. ALT is suggested to be most closely related to the amount of liver fat (Targher 2009), which is further underlined by the association of the I148 allele of the PNPLA3 gene (chromosome 22) with ALT. The I148 variant of the PNPLA3 gene is associated with hepatic fat content and fatty liver, and predisposes individuals to an 28% increase in ALT activity (Vernon et al. 2011). ALT, GGT, and (to a lesser degree) AST levels increase with increasing levels of BMI (Alatalo et al. 2008). Partial correlations taking alcohol consumption or BMI into account, show that ALT levels depend more on BMI, whereas GGT levels are most dependent on differences in ethanol intake (partial correlations for AST were not reported) (Alatalo et al. 2008).

The role of GGT as marker of oxidative stress is complex. Although GGT is involved in protecting the cell from oxidative stress by maintaining adequate levels of the intracellular

antioxidant glutathione, the presence of iron is suggested to trigger pro-oxidant effects of GGT (Lee et al. 2004). Thus on the one hand, increases in GGT can mark increases in glutathione that actually present anti-oxidant effects to protect the cell from oxidative stress during normal metabolism, e.g. that of alcohol. On the other hand, increases can indicate the presence of ROS. Indeed, higher levels of GGT were associated with higher levels of markers for oxidative stress (Lee et al. 2004; Yamada et al. 2006; Bo et al. 2005) and lower levels of serum anti-oxidants (Sugiura et al. 2005; Lee et al. 2004; Lim et al. 2004). In addition, GGT and ALT correlate positively with CRP, a marker of inflammation (Yamada et al. 2006; Kerner et al. 2005).

5. Aim and research question

Alcohol use, especially heavy alcohol use, is associated with several changes in the body, including liver injury. Liver enzyme levels are used as markers of liver injury, and likely reflect, at least partly, effects of alcohol-related injury. The exact nature of the association between alcohol use and liver enzyme levels is not yet entirely clear however. Not every drinker has elevated liver enzyme levels, which may reflect genetic differences among individuals causing individual differences in liver enzyme levels. If alcohol use is associated with increased liver enzyme levels, and both alcohol use and liver enzyme levels are genetically mediated, then the question arises whether, and if so, to what extent, the genetic epidemiology of alcohol use and liver enzyme levels is shared, which is the focus of this thesis.

This thesis takes a genetic perspective on alcohol use and liver enzyme levels to elucidate the association between them. It first describes what amount of the variance in alcohol use and liver enzyme levels, and the overlap among them, can be ascribed to genetic and environmental effects. These analyses are based on data from twins and their family members registered with the Netherlands Twin Register (NTR) (Boomsma et al. 2002c; Boomsma et al. 2006). Twin-family designs can determine the importance of genetic risk factors for alcohol use and liver enzyme levels, and the amount of genetic overlap in risk factors among these traits, by the fact that mono- and dizygotic twin pairs and their family members differ in genetic relatedness to different degrees. Next, genetic marker data are analyzed to estimate what part of the (twin-family based) heritability estimates for alcohol use and liver enzyme levels are attributable to assessed genetic marker data. It is then examined if the association between alcohol use and liver enzyme levels can be traced back to genetic marker data. These analyses are based on data from NTR individuals as well as from participants of the Netherlands Study on Depression and Anxiety (NESDA) (Penninx et al. 2008).

Furthermore, a gene finding study is performed to detect genetic risk loci that explain differences alcohol use.

6. Outline thesis

First, a method section explains the methodology that has been applied to investigate the genetic architecture of alcohol use and liver enzyme levels (**Chapter 2**). Chapters 3 to 8 can be subdivided into two parts. In the first part, studies are presented that rely on twin-family data to obtain estimates of the heritable component of variation in alcohol use and liver enzyme levels. In these studies the genetic relatedness between individuals is inferred and the influence of genetic effects is estimated based on the correlations between the phenotypic values of family members. In the second part of this thesis, studies are presented that make use of genetic marker data (single nucleotide polymorphisms, SNPs) with the aims of gene finding and studying the genetic architecture of alcohol use and liver enzyme levels explained by SNPs.

Chapter 3 studies the importance of genetic and environmental influences in explaining individual differences in alcohol intake. For this study, the resemblance in alcohol intake levels among twins pairs, their siblings and parents is analyzed to obtain an estimate of the heritability. Besides effects of genetic transmission, effects of cultural transmission from parents to offspring are tested. Does the level of alcohol intake by the parents affect that of their children, e.g. through social modeling, even when the transmission of the genetic risk affecting alcohol intake levels is taken into account?

Chapter 4 describes a study on the development of symptoms of alcohol abuse and dependence (AAD) from adolescence into young adulthood, an important timeframe for the development of alcohol use, and how this development can be explained. For this study, longitudinal data on the CAGE are analyzed (Ewing 1984), collected in twins aged 15-32 years old. Specifically, it is examined what the relative contribution is of genetic and environmental effects on the development of symptoms of AAD in adolescence and young adulthood. Can this development be explained by one set of genes, or do different genes get expressed along adolescence and young adulthood, for instance when individuals tend to leave the parental home?

Chapter 5 describes what part of individual differences in blood levels of the liver enzymes GGT, ALT and AST can be ascribed to genetic effects. By analyzing the resemblance in liver enzyme levels among twin- and sibling pairs and parents and offspring, an estimate on the heritability is obtained. By analyzing data from twins, siblings and parents, possible effects of spousal

resemblance on the heritability can be taken into account, and non-additive genetic effects and effects of shared environment can be estimated simultaneously.

Chapter 6 presents a study on the association of alcohol intake with GGT. The relation between alcohol intake levels and GGT among twins and their family members can inform the underlying mechanism of association. Do individuals have elevated GGT levels because they drink higher amounts of alcohol ? (i.e. alcohol intake causes increased GGT levels) Or is there a common cause, e.g. shared genetic factors that influence both alcohol consumption as well as variation in GGT levels which explains the association between alcohol use and GGT at the population level?

In **Chapter 7**, a candidate gene study is performed on the association of loci in the ADH gene cluster with measures of alcohol use. This gene cluster harbors functional variants in the ADH1B and ADH1C genes which have been associated with risk for alcoholism previously (Edenberg 2007). Relatively little is known about genetic variants in other ADH genes and how these are related to measures of alcohol use other than alcoholism however, which is the focus of this study.

Chapter 8 examines what part of the variance in liver enzyme levels and their covariance with alcohol use can be explained by genetic marker data. Two relatively novel methods are applied to estimate the heritability of alcohol use and liver enzyme levels based on SNPs (genetic marker data that differ from the DNA reference variant by a single base pair, A, C, T, G). A first method is based on the genetic relatedness among individuals implemented in the software package GCTA (Yang et al. 2011a) and a second method on density estimation (proposed by So et al. (2011)). In addition, it is explored if part of the variation in liver enzyme levels that can be explained by SNPs, is shared with that for alcohol use.

This thesis is concluded with a summary and general discussion (**Chapter 9**) and Dutch summary (**Chapter 10**).